Attorney Docket No. 11000.1037c3
PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of **Lorna Strachan, Matthew Sleeman, Nevin Abernethy, Rene Onrust, Krishanand D. Kumble, and James G. Murison**

Application No. : 09/823,038

Group Art Unit: 1646

Filed : March 28, 2001

For : **COMPOSITIONS ISOLATED FROM STROMAL CELLS AND METHODS FOR THEIR USE**

Examiner : Ruixiang Li

DECLARATION OF DR. J. GREG MURISON

MAIL STOP RCE
Commissioner for Patents
P.O. Box 1450
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The undersigned, Dr. J. Greg Murison, hereby declares:

1. I am presently Senior Staff Scientist at Genesis Research and Development Corporation Limited, the assignee of the subject patent application. I have a PhD in the field of Immunology. The following studies were carried out under my supervision.

2. The effects of administration of FGFR5β protein *in vivo* were examined in three experiments as follows.

Experiments 1 and 2

Experiment 1 used BALB/cByJ mice and experiment 2 used C3H/HeJ mice. Both sets of mice were injected subcutaneously with 5 ug (55 nM in 0.1 ml PBS) of murine FGFR5β extracellular domain (amino acids 1 - 373 of SEQ ID NO: 31 [Please confirm that this is correct]) - murine IgG3 [Please confirm that this was truly IgG3 and not IgG1 as used in Expt. 3] Fc fusion protein (prepared as described in Example 5 of the instant specification) in the morning and the same dose in the evening (i.e. each mouse received 10 ug per day) for five days. Control mice received PBS alone. On the sixth day, the mice were sacrificed and the draining lymph nodes (axillary and lateral axillary) were removed. A single cell suspension was generated from the lymph nodes of each mouse and the number of cells collected from each mouse was determine by trypan blue viability counting using a haemocytometer. The lymph node cells collected from the FGFR5-treated mice were then pooled. The lymph node cells collected from the PBS-treated mice were amalgamated into a separate pool of cells. The cells from both the FGFR5 and PBS-treated mice were then stained for the cell surface antigens listed in Table 1, below, and analyzed by flow cytometry.

Experiment 3

In this experiment, C3H/HeJ mice were injected subcutaneously with 10 ug (110 nM in 0.1 ml PBS) of murine FGFR5β ECD - human IgG1 Fc fusion protein in one injection per day for 5 days. While the treatment regime differed from that used in Experiments 1 and 2 above, the total dose of protein administered to the mice did not alter. Control mice were administered human IgG1 Fc fragments alone. On the sixth day, the mice were sacrificed and the draining lymph nodes (axillary and lateral axillary) removed. The number of cells collected from each mouse and the presence of cell surface antigens was determined as described above.

As shown in Table 1, *in vivo* administration of FGFR5 was found to stimulate lymphadenopathy, or enlargement of the lymph nodes. When compared to mice treated with Fc protein, the frequency of B cells doubled in the draining lymph nodes of FGFR5-

retained in the lymph nodes. The cells are, however, activated as there is an increase in the number of cells expressing the very early activation antigen, CD69.

Table 1: Comparison of 3 *in vivo* experiments testing the effects of soluble FGFR5 in mice

		Experiment 1		Experiment 2		Experiment 3	
		Balb/c		C3H/HeJ		C3H/HeJ	
Markers	Cell type recognized	Murine Fc FGFR5	PBS	Murine Fc FGFR5	PBS	Human Fc FGFR5	Human Fc
CD3	T cell	63	81	59	82	32	67
CD19	B cell	35	21	39	16	61	26
Class II	B cell and macrophage	41	20	ND*	ND	ND	ND
CD45R	B cell	ND	ND	ND	ND	72	31
CD69	Activated cells	23	14	18	10	21	10

* ND = Not determined

These results indicate that *in vivo* administration of FGFR5 polypeptide may be effectively employed to enhance an immune response.

3. The undersigned further declares that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful, false statements, and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 35 of the United States Code.

J. Greg Murison, Ph.D.

16 JUNE 2008
Date